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Molecular characterization, expression in Escherichia coli, and epitope analysis of a two EF-hand calcium-binding birch pollen allergen, Bet v 4.

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Birch pollen belongs to the most potent elicitors of Type I allergic reactions in early spring. Using serum IgE from a birch pollen allergic patient, two cDNA clones (clone 6 and clone 13) were isolated from a birch pollen expression cDNA library constructed in phage lambda gt11. Clone 6 encoded a 9.3 kD two EF-hand calcium-binding protein, designated Bet v 4, with significant end to end sequence homology to EF-hand calcium-binding allergens from weed and grass pollen. Recombinant Bet v 4, expressed as beta-galactosidase fusion protein, reacted with serum IgE from approximately 20% of pollen allergic individuals. Depletion of allergenbound calcium by EGTA treatment lead to a substantial reduction of IgE-binding to Bet v 4, indicating that protein-bound calcium is necessary for the maintenance of IgE-epitopes. The greatly reduced IgE-binding capacity of clone 13, a Bet v 4 fragment that lacked the 16 N-terminal amino acids, indicated that the N-terminus contributes significantly to the proteins IgE-binding capacity. By IgE-inhibition experiments it was demonstrated that recombinant Bet v 4 shared IgE-epitopes with natural Bet v 4 and a homologous timothy grass pollen allergen. Recombinant Bet v 4 may therefore be considered as a relevant crossreactive plant allergen, which may be used for diagnosis and treatment of patients suffering from multivalent plant allergies.

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